Genetic variations in *TAOK1, TMTC4, NTF3,* and *CNTN1* and their association with noise-induced hearing loss

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Abstract

Noise-induced hearing loss (NIHL) affects more than 10% of the adult population in developed countries, especially those with high noise levels. This study's objective is to examine the effect of the variability in sensitivity to noise exposure of genetic variations in the *TAOK1, TMTC4, NTF3,* and *CNTN1* genes. A total of 323 people in seven different businesses were included in the scope of the research. SNP genotyping was performed over a total of eight SNP regions, including SNP regions from the *TAOK1, TMTC4, NTF3,* and *CNTN1* genes, using the ABI 7500 Real-Time PCR System with 96 wells according to the rs191010498 *TAOK1* C/T, rs1469121309 *TAOK1* A/T, rs763560019 *TMTC4* T/A, rs1484543954 *TMTC4* G/C, rs1334049414 *NTF3* T/C, rs1438314793 *NTF3* G/A, rs762307549 *CNTN1* G/A,T, and rs745850349 *CNTN1* T/A Custom TaqMan® SNP Genotyping Assays and standard protocols. The CT heterozygous frequencies of the *TAOK1* gene were observed, and age, working time, and working in a noisy environment over 85 dBA ($p < 0.05$) were found to be significantly higher in the hearing loss group compared to those in the control group. In the Turkish population, sensitivity to noise-induced hearing loss was observed in five out of eight SNP regions of the *TAOK1, TMTC4, NTF3,* and *CNTN1* genes, with *TAOK1, TMTC4,* and *CNTN1* genes also being associated with hearing loss. However, no statistically significant difference was found in the *NTF3* genes regarding rare variants. The results show the *TAOK1*-rs191010498 (p = 0.01), *TAOK1*-rs1469121309 (p = 0.03), *TMTC4*-rs763560019 (p = 0.01), *CNTN1*-rs762307549 (p = 0.01), and *CNTN1*-rs745850349 (p = 0.03) polymorphisms to be

associated with NIHL. Identifying *TAOK1, TMTC4, CNTN1* polymorphisms is important in terms of providing a new contribution to the genetic roles associated with hearing loss.

Keywords: NIHL, SNP genotyping assay, variation, gene, polymorphism

1. Introduction

Hearing loss partially originates from noise, one of the most harmful physical factors to health (Tessier-Sherman et al., 2017; Münzel et al., 2020; Wang et al., 2018). Globally, around 16% of adults with debilitating hearing loss suffer from noise exposure at work (Themann et al., 2019). Noise-induced hearing loss (NIHL) is a form of sensorineural hearing loss occurring as an occupational disease observed among employees as a result of chronic exposure to high frequencies (between 3-6 kHz) and noisy environments (Tessier-Sherman et al., 2017). NIHL is caused by a combination of environmental factors as well as genes that influence sensitivity (Miao et al., 2019). NIHL is most likely caused by long-term exposure to noises at an intensity and frequency that harm cochlear epithelial cells. A higher level of noise in the workplace (8h of 85 dBA) results in the cochlea absorbing excess energy, followed by the production of free radicals (Hu & Deng, 2014). Free radicals cannot be neutralized by the antioxidant system, so damage occurs in the cochlear sensory epithelium (Ohlemiller et al., 1999). Hence, genes that regulate reactive oxygen types including superoxide dismutase, glutathione S-transferase, and catalase, might influence cochlear sensitivity to NIHL (Reddy et al., 2001; Shen et al., 2012).

Vocational noise is a common occupational hazard for employees, and NIHL is the most common type of sensorineural hearing loss, as well as age-related hearing loss worldwide (Konings et al., 2009). NIHL has been confirmed as a multi-factor disease caused by the interaction of both genetic and environmental factors (Carlsson et al., 2005). Although NIHL is clearly known to be related to noise, studies continue with regard to their genetic role. Internal ear cell apoptosis or direct mechanical damage to metabolic products and cochlea structures during signal transmission may cause pathogenesis (Henderson et al., 2006; Le Prell et al., 2003; Le Prell et al., 2007; Henderson et al., 1993). Despite this, numerous population studies have shown each individual to be susceptible to NIHL in varying degrees despite being exposed to the same level of noise density (Konings et al., 2009; Carlsson et al., 2005; Henderson et al., 2006; Le Prell et al., 2003; Le Prell et al., 2007; Henderson et al., 1993; White et al., 2009). Genetic variations also contribute to the development of NIHL in animals (Davis et al., 2001; Kowalski et al., 2014). The *HSP70, EYA4, CDH23, GRHL2,* and *DFNA5* genes contain single nucleotide polymorphisms (SNPs) that have been found to influence a person's genetic susceptibility to noise-induced and occupational hearing loss (Chang et al., 2011; Zhang et al., 2015). The interaction between workers' genetic predisposition and environmental factors can play a significant role in NIHL.

Multi-factor interactions cause irreversible damage with regard to NIHL, and the STAT3 protein is an essential signal transduction and transcription factor. The rs1053023 and rs1053005 polymorphisms were found to be associated with NIHL and smoking, with alcohol use also somewhat affecting susceptibility to NIHL. This suggests that the rs1053023 and rs1053005 C alleles of *STAT3* may play a crucial role in the emergence of NIHL and that NIHL may be a susceptibility biomarker for Chinese workers (Gao et al., 2020).

Grainyhead-like 2 *(GRHL2)* is a transcription factor that affects the composition of the cortex. Three members of the grainyhead-like transcription factor family *(GRHL1, GRHL2,* and *GRHL3)*

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play a role in epithelial adhesion regulation. The cells lining the cochlear duct express high levels of *GRHL2,* which maintains epithelial cells and promotes embryonic development. The *GRHL2* gene is located on chromosome 8q22.3 and contains 15 introns and 16 exons. Liu et al. (2020) found the *GRHL2* rs3735715G > A polymorphism in the dominant pattern (GA/AA vs. GG) and allele pattern (G allele vs. A allele) to be associated with NIHL risk in NIHL cases and controls.

This study assesses whether the genetic variability of the thousand-and-one kinase 1 *(TAOK1),* transmembrane O-mannosyltransferase targeting cadherins 4 *(TMTC4),* neurotrophin 3 *(NTF3),* and contactin 1 *(CNTN1)* are related to hearing loss in the Turkish population in order to evaluate the interaction between genetic predisposition and environmental factors and to evaluate its relationship with NIHL.

2. Materials and Methods

2.1 Sample Size and Study Power

Power estimation is an essential part of genetic association studies and is used to identify candidate genes that contribute to NIHR susceptibility. Power calculations for the current study were made using the program QUANTO version 1.2 4 (http://hydra.usc.edu/gxe/) with the following options: an unmatched case-control study design; a 29% population frequency for NIHR (Nelson et al.,2005); rs191010498, rs1469121309, rs763560019, rs1484543954, rs1334049414, rs1438314793, rs762307549, and rs745850349 SNPs (with Type I Error 0.05, Type II Error 0.20, Power 0.80); C, A, G, T-allele frequency of 0.80; and an inherited recessive genetic mode. Based on our predictions, our study calculated a minimal sample size of 148 subjects each for the control and NIHR groups. Our study was carried out on 300 people, including 150 NIHR and 150 controls.

The research was carried out on 150 workers working in seven enterprises in metal casting production, packaging, and textile industries at Eyüp and organized industrial sites in Halkalı. The measurements were carried out between September 2019 and June 2020. Informed consent was obtained from each participant prior to the research. Ethics committee permit #287 dated 18/03/2019 was obtained from the Clinical Research Ethics Committee at Istanbul Medical Faculty.

Prior to pure sound audiometry, the people included in the study were questioned about their previous work history, smoking, alcohol use, and habitual drug use. Employees with diseases that may affect their hearing thresholds and those who have a history of ototoxic drug use, chronic disease or a family history of deafness, ear infections, and hearing loss due to other reasons (e.g., explosives, heavy metals) were excluded from the study.

2.2. Noise Measurements and Audiological Assessments in the Work Environment

The AD226 audiometer (Interacoustics AS Company, Denmark) was used for measurements at frequencies of 0.5, 1, 2, 4, and 8 kHz for audiometric examinations of both ears. All audiometry tests were performed using the audiometer in a vehicle with a standard quiet cabin. To reduce the effect of temporary threshold shifts in all participants before audiometry, care was taken to measure them at least 16 hours before entering the work environment in the morning hours.

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In order to determine hearing loss in the audiometric stage by conducting airway pure-sound audiometric tests, the arithmetic average of the hearing values were taken into account at 500, 1000, and 2000 Hz and the hearing threshold values at 4000 Hz, which is the most sensitive region in acoustic trauma. The results were arranged by age (The Ministry of Labor and Social Security General Directorate of Occupational Health and Safety, 2012).

Noise measurements in the workplace were measured using the B&K 2250 device. The noise statistical analyzer was adjusted to fit the ear height using a microphone pointing at the source of noise (approximately 1.5 m while standing). Each location was measured three times, and the average of the individual's noise exposure levels was recorded. Equivalent continuous dBA-weighted sound pressure levels (LAeq,8h) were evaluated. Noise measurement was done between 8:00 a.m. and 4;00 p.m. Noise levels in the workplace were evaluated using the Bruel-Kjear 2250 Mediator Integrating Sound Level Meter; LAeq was evaluated with regard to LAFmin and LAFmax. LAeq refers to the equivalent noise level obtained during the measurement time; LAFmax refers to the highest sound pressure value obtained during the measurement time; LAFmin refers to the lowest sound pressure level value obtained during the measurement time.

2.3. DNA Isolation and SNP Genotyping

A venous blood sample of was taken from each individual in the patient and control groups in a 3 mL EDTA tube for genomic DNA extraction. Genomic DNA was extracted from the blood samples using the PureLink™ Genomic DNA Mini Kit. SNP information was obtained from the dbSNP database (https://www.ncbi.nlm.nih.gov/snp/).

The study chose potential functional regions (intronic, missense) because they have a higher chance of being causal SNP. The SNP regions included in the study were chosen as the rare SNPs that have not previously been examined in the literature. The eight SNP regions are the rs191010498 (SNP1; intronic variant), rs1469121309 (SNP2; missense variant), rs763560019 (SNP3; missense variant), rs1484543954 (SNP4; missense variant), rs1334049414 (SNP5; missense variant), rs1438314793 (SNP6; intronic variant), rs762307549 (SNP7; intronic variant), and rs745850349 (SNP8; missense variant). SNP genotyping was performed for these eight SNP regions using the Custom TaqMan® SNP Genotyping Assay.

The Custom TaqMan ® SNP Genotyping Assay method employed VIC and FAM dye for fluorescent emission spectra. Amplifications and analyses were carried out using the ABI 7500 Real Time PCR System with the 384 bore format according to the standard protocols (Applied Biosystems). Analyses of the participants' 11:g.29451055C>T genotype (rs:191010498), 11:g.29451616A>T genotype (rs:1469121309), 11:g.100605046T>A genotype (rs:763560019), 11:g.100626145G>C genotype (rs:1484543954), 12:g.5494369T>C genotype (rs:1334049414), 12:g.5432382G>A genotype (rs:1438314793), 12:g.40918635G>A genotype (rs:762307549), and 12:g.40908459T>A genotype (rs:745850349) were respectively carried out with the Applied Biosystems 7500 Real-Time PCR System using Custom TaqMan™ SNP Genotyping Assay through SNP1 ID assay No:ANMF6ZT, SNP2 ID assay No: ANNKZKP, SNP3 ID assay No:ANPR46M, SNP4 ID assay No: ANRWNRJ, SNP5 ID assay No: ANT2HCG, SNP6 ID assay No: ANU7CWE, SNP7 ID assay No:ANWC6GC, and SNP8 ID assay No:ANXGYZ9 (applied biosystems) on the Step One Real-time PCR system.

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The choice of SNPs in our study is based on the criteria of being rare SNPs of low frequency (minor allele frequency [MAF] < 0.05) for which no prior studies on experimental polymorphisms associated with NIHL have been performed. The analysis of the SNPs from the NCBI dbSNP database shows that most of the confirmed SNPs in the human genome are rare.

When considering the distributions of SNPs by MAF in the International HapMap Database (http://hapmap.ncbi.nlm.nih.gov/), the MAF values of the eight SNP regions included in our study increased sharply when moving from MAF = 0.5 to MAF ≤ 0.002. The chosen SNPs covered both coding region (missense variant) as well as non-coding region (intron variant).

We think that rare polymorphisms play an important role in determining the genetic mechanisms underlying many common diseases. Because multiple genes may underlie NIHL susceptibility, a significant proportion of affected individuals can be expected to have the rare genetic polymorphism associated with hearing loss.

Our study's determination that the distribution in all SNPs is consistent with the Hardy-Weinberg (HWE) distribution raises the possibility that rare alleles may be more functional than the effects of common variants. In line with these data, having future research as to the polymorphism focus on cases with more severe hearing loss may be beneficial for detecting rare SNPs associated with NIHL.

2.4. Statistical Analysis

The continuous data obtained within the scope of the study were presented as standard deviations as well as the mean ones and intermittent data presented as frequencies and percentages. A single-sample Kolmogorov-Smirnov test was performed to test whether the variables obtained within the scope of the study conform to a normal distribution. To compare continuous variables between the groups, the t-test was used in independent groups for the normally distributed variables, and the Mann-Whitney U test was used for the non-normally distributed variables. The Hardy-Weinberg (HW) distribution and allelic frequencies of the analyzed genes were calculated for each group. The frequency of polymorphism among the groups was compared with the chi-square test and Fisher's precise test, and odds ratios were calculated at a corresponding 95% confidence interval. Logistic regression analysis was applied to evaluate the independent effects of the factors causing hearing loss by regarding hearing loss as a dependent variable (Forward LR). Statistical significance was established at $p \leq 0.05$ is considered two-way.

3. Results

Individuals were divided into two groups based on the audiometry results after considering the inclusion and exclusion criteria. Of the 162 NIHL cases, 150 individuals who might have NIHL were considered as the case group. Twelve members from the hearing loss group were excluded from the assessment due to a history of COVID, diabetes mellitus, hearing loss due to infection, and/or hereditary disease. Of the 161 people unlikely to have NIHL, 150 were included in the study as individuals with normal hearing. Eleven people were excluded from the assessment due to a history of COVID, diabetes mellitus, and/or hypertension. The average daily working time in all workplaces is 8 hours. Employees used personal protective equipment, and each business received

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services from its partner health and safety unit. Each business had a workplace physician and occupational safety specialist.

The linkage disequilibrium (LD) patterns of SNPs were measured using r^2 and D' values with the program Ensembl. Two SNPs (rs191010498, rs1469121309) were identified in *TAOK1,* as well as two SNPs (rs763560019, rs1484543954) in *TMTC4,* two SNPs (rs1334049414, rs1438314793) in *NTF3,* and two SNPs (rs762307549, rs745850349) in *CNTN1*.

Table 1 provides the medians as well as lowest and highest noise levels (dBA) of the workplace noise measurements. While the equivalent noise levels measured in four factories in the metal business ranged from 93.4 to 96.7 dBA, these levels were measured between 80.4 and 82.1 dBA in two factories from the textile business and as 81.1 dBA in the packaging business.

Table 1. *Workplace Noise Measurements*

Workplace	***Mdn***	**Min**	**Max**
Packaging	81.1	68.8	91.9
Textile-1	80.4	69.6	92.5
Textile-2	82.1	80.8	87.4
Metal-1	93.4	78.1	99.9
Metal-2	94.7	84.5	112.5
Metal-3	96.7	80.2	105.5
Metal-4	95.0	83.0	103.9

Table 2 provides the distributions for those considered to have NIHL.

Table 2. *Audiometric Test Results by Groups*

Workplace	**Number of Employees**	**NIHR (acoustic notch)**	**Percentage**	**Normal Hearing**	**Percentage**
Packaging	38	10	0.26	28	0.74
Textile-1	18	10	0.56	8	0.44
Textile-2	27	8	0.30	19	0.70
Metal-1	118	70	0.59	48	0.41
Metal-2	27	15	0.56	12	0.44
Metal-3	28	13	0.46	15	0.54
Metal-4	44	24	0.55	20	0.45
Total	300	150		150	

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Table 3 provides the distributions for hearing thresholds at different frequencies in the NIHL and control groups. When analyzing the average values of people considered to have NIHL at 0.5, 1, 2, 4 and 8kHZ, the acoustic notch being higher than 25 at 4kHZ is remarkable.

Table 3. *The Distribution of Hearing Thresholds at Different Frequencies in the NIHL and Control Groups*

Hearing loss group	**0.5kHz**	**1kHz**	**2kHz**	**4kHz**	**8kHz**
Ear	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)
Right ear	19.50 (5.26)	20.5 (6.20)	35.42 (7.57)	42.34 (14.06)	34.5 (6.86)
Left ear	19.20 (7.35)	20.4 (6.15)	36.19 (9.36)	41.13 (12.55)	33.89 (6.89)
Control group	0.5Kz	1Khz	2Khz	4Hkz	8Khz
	M (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)
Right ear	16.20 (5.75)	17.80 (5.20)	16.70 (7.35)	17.30 (8.50)	18.56 (7.10)
Left ear	16.55 (5.26)	17.60 (5.80)	17.25 (6.75)	18.30 (6.50)	17.50 (8.20)

M: Mean; SD: Standard deviation

Table 4 shows the demographic characteristics by groups. Examining Table 4 shows age and work times to be significantly higher in the hearing loss group than those in the control group ($p < 0.001$). Height, weight, and BMI values are not statistically significant ($p > 0.05$). When examining the gender distributions of the groups, the ratio of males to females is found to be n_{female} = 133 (89%) and n_{male} = 17 (11%) in the hearing loss group and n_{female} = 123 (82%), n_{male} = 23 (15%) in the control group. This difference was not found to be statistically significant ($X^2 = 1.38$; $p = 0.30$).

Table 4. *Demographics by Group*

	Group	***N***	***M***	***SD***	***Mdn***	**Significance (*t*; *z*)**	***p***
Age	Hearing loss	150	40.71	6.80	41	*t* = 7.21	< 0.001
	Control	150	35.80	5.05	35.71		
Height	Hearing loss	150	172.73	5.97	172	*t* = 0.04	0.96
	Control	150	172.76	6.94	173		
Weight	Hearing loss	150	77.39	10.63	78	*t* = 0.5	0.61
	Control	150	78.03	11.28	78.5		
BKI	Hearing loss	150	25.95	3.47	25.85	*t* = 0.45	0.64
	Control	150	26.13	3.44	26.28		
Operating Time	Hearing loss	150	13.08	10.63	10	*z* = 4.29	< 0.001
	Control	150	8.20	7.75	6		

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Table 5 presents the genotype and allele frequency distributions for the SNPs investigated in the *TAOK1, TMTC4, NTF3,* and *CNTN1* genes as well as their relationship with NIHL.

Table 5. *Genotype and Allele Frequency Distribution of SNPs Investigated in the TAOK1, TMTC4, NTF3, and CNTN1 Genes and Their Relationship with Noise-Induced Hearing Loss*

SNP	Geno type	Hearing loss *n*	(%)	Control *n*	(%)	*OR* 95% CI for Genotype	χ2 Fisher's Exact test (p)	HW NIHR	HW Control	Allele frequencies -NIHR (%); *n* Major	Minor	Allele frequencies-Control (%); *n* Major	Minor	χ2 Fisher's Exact test (*p*)	*OR* 95% CI for Allele frequencies
										C	T	C	T		
SNP1- TAOK1-rs191010498	C/C	138	0.92	148	0.99	6.43	0.01	χ2=0.26	χ2=0.001	0.96	0.04	0.99	0.01	0.12	6.2
(MAF: T: 0.002995/15)	C/T	12	0.08	2	0.01	(1.41-29.28)		*p*=0.60	*p*=0.96	144	6	149	1		0.79-52.23
	T/T	-		-											
										A	T	A	T		
SNP2- TAOK1-rs1469121309	A/A	140	0.93	148	0.99	5.28	0.03	χ2=0.17	χ2=0.06	0.97	0.03	0.99	0.01	0.21	5.13
(MAF: T: 0.000004/1)	A/T	10	0.07	2	0.01	(1.13-24.55)		*p*=0.67	*p*=0.93	145	5	149	1		0.59-44.53
	T/T	-		-											
										T	A	T	A		
SNP3-TMTC4-rs763560019	T/T	141	0.94	149	0.99	9,51	0.01	χ2=0.14	χ2=0.001	0.97	0.03	1.00	0.00	0.21	5.13
(MAF: A: 0.000056/14)	T/A	9	0.06	1	0.01	(1.18-76.07)		*p*=0.70	*p*=0.96	145	5	149	1		0.59-44.53
	A/A	-		-											
SNP4-TMTC4-rs1484543954	G/G	-		-						C	G	C	G		
(MAF: C: 0.0005/1)	G/C	-		-											
	C/C	150	1.00	150	1.00	NC	NC	NC	NC	NC	NC	NC	NC		NC
										T	C	T	C		
SNP5-NTF3-rs1334049414	T/T	150	1.00	150	1.00	NC	NC	NC	NC	NC	NC	NC	NC		NC
(MAF: C: 0.000004/1)	T/C	-		-											
	C/C	-		-											
										G	A	G	A		
SNP6-NTF3-rs1438314793	G/G	139	0.93	145	0.97	1,40	0.19	χ2=0.21	χ2=0,04	0.96	0.04	0.98	0.02	0.49	2.04
(MAF: A: 0.000004/1)	G/A	11	0.07	5	0.03	(0.98-1.99)		*p*=0.64	*p*=0.83	144	6	147	3		0.50-8.32
	A/A	-		-											
										G	A	G	A		
SNP7-CNTN1-rs762307549	G/G	141	0.94	149	0.99	9,51	0.01	χ2=0.14	χ2=0.001	0.97	0.03	1.00	0.00	0.06	11.37
(MAF: A: 0.00002/5)	G/A	9	0.06	1	0.01	(1.18-76.07)		*p*=0.70	*p*=0.96	145	5	150	0		0.62-207.77
	A/A	-		-											
										T	A	T	A		
SNP8-CNTN1-rs745850349	T/T	140	0.93	148	0.99	5,28	0.03	χ2=0.17	χ2=0.06	0.97	0.03	0.99	0.01	0.21	5.13
(MAF: A: 0.000008/2)	T/A	10	0.07	2	0.01	(1.13-24.55)		P=0.67	P=0.93	145	5	149	1		0.59-44.53
	A/A	-		-											

OR = Odds Ratio; NC = not calculatable.

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The HW distributions for the SNPs investigated in the *TAOK1, TMTC4, NTF3,* and *CNTN1* genes were examined in both the hearing loss group and the control group. As a result, the distributions for all SNPs were observed to be in accordance with the HWE distribution. The genotype and frequency for SNP1-rs191010498 in the *TAOK1* gene were calculated to be 92% (C/C) and 8% (C/T) in the hearing loss group, and 98.6% (C/C) and 1.4% (C/T) in the control group. An *OR* = 6.43 (1.41-29.28) was found between the two groups. For the SNP1 (C/T), the trend towards higher NIHL was found to be statistically significant ($p = 0.01$). The T/T genotype was not observed in SNP1-rs191010498. When calculating the allele frequencies for SNP1-rs191010498, the C allele was found to be 96% in the NIHR group with 4% for the T allele, while 99% and 1% in the control group respectively, with an *OR* = 6.2 (95% CI [0.79, 52.23])

For the *TAOK1* gene, SNP2- rs1469121309 was calculated to be 93.3% (A/A) and 6.7% (A/T) in the hearing loss group and 98.6% (A/A) and 1.4% (A/T) in the control group, with an *OR* = 5.28 (1.13-24.55) being found between the two groups. For SNP2 (A/T), the trend toward higher NIHL was found to be statistically significant ($p = 0.03$). The T/T genotype was not observed in SNP2-rs1469121309. When calculating the allele frequencies for SNP2- rs1469121309 in the *TAOK1* gene, the A allele was found to be 97% and the T allele to be 3% in the NIHR group, while 99% and 1% for the control group respectively, with an *OR* = 5.13 (95% CI [0.59, 44.53]).

According to the results obtained regarding SNP3- rs763560019 in the *TMTC4* gene, T/T was 94% and T/A was 6% in the hearing loss group, while T/T was 99.4% and T/A was 0.6% in the control group. An OR = 9.51 (1.18-76.07) was found between the two groups. For SNP3 (T/A), a trend toward higher NIHL was found to be statistically significant ($p = 0.01$). A/A was not observed for SNP3-rs763560019 in the *TMTC4* gene. When calculating the allele frequencies regarding SNP3-rs763560019, the T allele was calculated to be 97% and the A allele to be 3% in the NIHR group, while calculated to be 99% and 1% in the control group respectively, with an *OR* = 5.13 (95% CI [0.59, 44.53]). Allele frequency percentages for SNP2-rs1469121309 in the *TAOK1* gene are similar to those for SNP3-rs763560019 in the *TMC4* gene.

G/G and G/C genotypes were not observed for SNP4-rs1484543954 in the *TMTC4* gene, which was instead determined to be the C/C genotype for all individuals in both the patient and control groups. T/C and C/C genotypes were not observed for SNP5-rs1334049414 in the *NTF3* gene, with the T/T genotype being determined to be present for all individuals in the hearing loss and control groups.

The genotype frequency for SNP6-rs1438314793 in the *NTF3* gene was calculated to be 92.6% for G/G and 7.4% for G/A in the patient group and 96.7 % for G/G and 3.3% for G/A in the control group. The A/A genotype was not observed in SNP6-rs1438314793. An *OR* = 1.40 (0.98-1.99) was calculated between the two groups. Although a tendency for SNP6 to increase was found for the hearing loss group, the result was not statistically significant ($p = 0.19$). When calculating the allele frequencies for in SNP6-rs1438314793 in the *NTF3* gene, G allele was 96% and A allele was 4% in the NIHR group, and 98% and 2% in the control group respectively, with an *OR* = 2.04 (95% CI [0.50, 8.32]).

The genotype frequency for SNP7-rs762307549 in the *CNTN1* gene was calculated to be 94% for G/G and 6% for G/A in the patient group, and 99.4% for G/G and 0.6% for G/A in the control group. The A/A genotype was not observed in SNP7-rs762307549. For SNP7, a trend toward

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higher hearing loss by genotype was found to be significant, with an *OR* = 9.51 (1.18-76.07; p = 0.01). The genotype frequency for SNP8-rs745850349 in the *CNTN1* gene was calculated to be 93.3% for T/T and 6.7% for T/A in the hearing loss group, and 98.6% for T/T and 1.4% for T/A in the control group. The A/A genotype was not observed in SNP8-rs745850349. Meanwhile, an *OR* = 5.28 (1.13-24.55) was calculated between the two groups, and this difference was found to be statistically significant (p = 0.03). Of all the SNPs examined in the study, while a 100% homozygous distribution was observed for only SNP4 and SNP5, not very high frequencies were detected in the heterozygous distributions from the hearing loss group for the other SNPs (SNP1, SNP2, SNP3, SNP6, SNP7, and SNP8). These frequencies increased in favor of the hearing loss group. The existing differences were found to be statistically significant. When calculating the allele frequencies for SNP7-rs1438314793 in the *CNTN1* gene, the G allele was 97% and the A allele was 3% for the NIHR group, and 100% and 0% for the control group respectively, with an *OR* = 11.37 (95% CI [0.62, 207.77]).

When calculating the allele frequencies for SNP8-rs745850349 in the *CNTN1* gene, the T allele was 97% and the A allele was 3% for the NIHR group, and 100% and 0% for the control group respectively, with an *OR* = 5.13 (95% CI [0.59, 44.53]). The major allele frequencies in the significant SNPs ranged from 96% to 100%, while the minor allele frequencies ranged from 1% to 5%.

Table 6 presents the logistic regression analysis of the variables affecting NIHL (e.g., age; working time; smoking status; ambient noise measurement; *TAOK1, TMTC4,* and *CNTN1* genes) and the results of the logistic regression model for those with and without hearing loss.

Table 6. *Examination of Variables Affecting NIHL with Logistical Regression*

Independent variables	**B**	***SE***	**Wald**	***df***	**Sig.**	***OR*** *	**95% CI for *OR***	
							Lower	Upper
TAOK1 (Ref:CC)						1		
TAOK1(CT)	1.828	0.788	5.376	1	0.02	6.219	1.327	29.146
Noise (ref < 85dBA)						1		
Noise (> 85dBA)	0.578	0.248	5.43	1	0.02	1.782	1.096	2.898
Age ref. (< 45)						1		
Age (> 45)	1.391	0.581	5.736	1	0.017	4.018	1.287	12.539
Working period (ref < 10 years)						1		
Working period (> 10 years)	0.724	0.268	7.328	1	0.007	2.063	1.221	3.485
Constant	-0.719	0.203	12.559	1	< 0.001	0.487		

When examining the variables entered into the model, people working in workplaces with noise levels greater than 85 dBA are 1.78 (95% CI [1.09, 2.89]) times more likely to have hearing loss; this increases to 2.06 times more (95% CI [1.22, 3.48] for those who have worked in a noisy environment for more than 10 years. The probability of hearing loss in those with a C/T pair in the

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TAOK1 gene (SNP1-rs191010498) is *OR* = 6.21 (95% CI [1.32, 29.14]) times higher. The probability of hearing loss in people over the age of 45 was found to be *OR* = 4.01 (95% CI [1.28, 12.53]) times higher. Noise exposure and working time in a noisy environment are important factors in industrial hearing loss. In addition, genetic variations in *TAOK1, TMTC4,* and *CNTN1* can also be considered important factors.

4. Discussion

Several components of the auditory system may be affected by sensorineural hearing loss, such as the Organ of Corti in the inner ear, the auditory nerve, and the auditory cortex. Drug-induced hearing loss, presbycusis, hereditary hearing loss, and NIHL are examples of hearing impairment resulting in a loss of sound perception and of analytical skills (Chen et al., 2022). Hearing loss is the most prevalent sensory impairment around the world and is frequently caused by noise. NIHL affects millions of people worldwide, with an estimated 7%-21% of adults with impaired hearing have been affected by occupational hearing loss (Nelson et al., 2005).

NIHL is the second most common form of sensorineural hearing loss after presbycusis. Noise levels are typically measured in decibels (dB) using the A-frequency weighting network (dBA), which indicates the risk of NIHL (Chen et al., 2022).

The genes involved in NIHL mainly occur in the structural integrity of the stereocilia (mechano-sensing organelles of the cochlear hair cells), ion homeostasis (pumps, channels and connexins), protective pathways (glucocorticoids and stress response) oxidative stress, and other processes. The paraoxonase2 genes go by *PON1, PON2,* and *PON3* and reside on the q arm of chromosome 7 (q21.3-22.1). These genes code for esterase enzymes. The *PON2* gene produces antioxidant effects throughout the body, while any changes in its sequence can increase reactive-oxygen species (ROS) and damage cochlear cells. The presence of polymorphisms in the *PON2* gene's rs12026 and rs7785846 in 94 male workers exposed to noise was associated with NIHL in Italy (Ahsan et al., 2020).

ATP2B2 may be able to serve as an early warning gene for NIHL, with low expression of this gene being able to lead to neurodevelopmental defects in the auditory system and hearing loss. Zhang et al. (2019) conducted a case-control study on 760 Chinese textile workers to investigate the relationship between *ATP2B2* polymorphisms and NIHL susceptibility. They found the genetic polymorphism of rs3209637 C within *ATP2B2* to be a risk factor for NIHL among Chinese workers and rs3209637 C to perhaps be a potential biomarker for NIHL patients.

The treatment of deafness could rely on the interaction of ion channels using auditory functions. A significant association exists between genetic hearing loss and the ion channel genes *KCNQ1, KCNE1, KCNQ4, P2RX2, TMC1, KCNJ10,* and *CACNA1D*. Emphasizing how the mutations that affect ion channel function in these genes can result in hereditary hearing loss is important due to their role in cochlear hair cells and auditory pathways (Zheng et al., 2021).

High temperature, ototoxic drugs, physiological stress, and noise stimulate the expression of heat shock proteins (HSPs). HSP70-1, HSP70-2, and HSP70-hom are all members of the *HSP70* gene family. A number of studies have shown HSP70 to produce a protective effect on hair cells by countering the ototoxic effects of chemicals, such as cisplatin and aminoglycosides. Lei et al.'s (2017) meta-analytic study comprehensively and systematically evaluated the association between

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HSP70 gene polymorphisms and NIHL susceptibility. Their results showed rs1043618, rs2075800, and rs2763979 polymorphisms not to be associated with susceptibility to NIHL in Caucasian men, while rs1061581 and rs2227956 polymorphisms were significantly associated with NIHL. NIHL was more prevalent among Caucasian individuals carrying the G allele. Further, rs2227956 was found to have a significant correlation with NIHL in all gene models in Caucasian individuals, with white individuals carrying the T allele as to be most at risk of NIHL.

Among humans' most interesting genes is *CDH23*, which encodes cadherin, which is a component of stereocilia tip links and which is associated with hair cell mechanotransduction channels. Mutations in *CDH23* in humans lead to both non-syndromic and syndromic hearing loss. According to Jiao et al. (2021), whose study had 776 subjects in the first phase and 1,117 subjects in Phase I+II, NIHL risk during Phase I was significantly higher for subjects carrying AA genotypes in rs3802711 compared to those carrying GG genotypes. Based on rs11592462 as well, subjects carrying the GG genotype showed a significantly higher risk of NIHL.

The superoxide dismutase 1 *(SOD1)* gene is associated with NIHL, and its activity is influenced by noise exposure. Liu et al. (2010) examined NIHL susceptibility in the Chinese population by investigating polymorphisms in the CuZn-superoxide dismutase gene. Their study genotyped four SNPs in the introns of *SOD1* and investigated their effects and interactions with noise exposure. The AA genotype in rs2070424 protected against NIHL, while the GG genotype in rs10432782 was found to be a genotype at-risk. The GG genotype in the SNP rs10432782 of *SOD1* has significantly higher SOD1 activity but lower malondialdehyde levels than the TT genotype.

Occupational NIHL is mainly caused by age, exposure time, noise level, and temporal structure of noise. Unstable noise levels must be reduced as a means of preventing permanent hearing damage in employees. This study found the probability of NIHL in those exposed to noise above 85 dBA to be OR = 1.78 (95% CI [1.09, 2.89]), with the OR being 2.06 (95% CI [1.22, 3.48]) times higher in those who worked in a noisy environment for more than 10 years. These findings coincide with research results regarding NIHL in the literature.

This study aims to assess whether the genetic variability of *TAOK1, TMTC4, NTF3,* and *CNTN1* is related to sensitivity regarding NIHL in the Turkish population. Many factors including genetic and environmental ones appear to play a role in NIHL. People who suffer from hearing loss after exposure to noise are frequently also exposed to multiple risk factors that interact with each other or that have been inherited (Rabinowitz et al., 2002).

Myocardial and cerebral infarctions result in an increase in mean platelet volume (MPV), which is an independent and strong predictor of mortality and morbidity following an event. Cell contraction, membrane blebbing, and the formation of apoptotic bodies are all associated with the activation of c-Jun N-terminal kinase by *TAOK1*. Meisinger et al. (2009) performed a genome-wide association study (GWAS; the KORA F3 500K study) and found MPV to be strongly associated with rs2138852 (HW = 1.000, p = 0.00000331) located upstream of *TAOK1* on chromosome 17q11.2. Their study showed SNP rs2138852 within the *TAOK1* gene region while our study showed the SNPs rs763560019 and rs2138852 in the *TAOK1* gene and the *TMTC4* gene's SNP rs1484543954 to have full linkage equilibrium.

An acoustic trauma study conducted by Patel et al. (2013; as cited in Fang et al., 2020, p. 11) observed the cochlear cells of rats traumatized by noise to express downregulation of miRNA-183 and upregulation of *TAOK1*; this may mean miR-183/*TAOK1* is likely to play a role in sensory

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hearing loss. The current study found the tendency for the *TAOK1* gene (SNP1- rs191010498 and SNP2- rs1469121309) to increase NIHL, which is statistically significant ($p = 0.01$).

A new O-mannosylation pathway involves the human proteins *TMTC1, TMTC2, TMTC3,* and *TMTC4.* Human TMTC proteins consist of four paralogues: *TMTC1* (Q8IUR5, 882 AA), *TMTC2* (Q8N394, 830 AA), *TMTC3* (Q6ZXV5, 915 AA), and *TMTC4* (Q5T4D3, 741 AA). An inactive form of *TMTC4* in mice results in a loss of hearing in early postnatal stages. Eisenhaber et al.'s (2021) transcriptome-wide association study showed *TMTC4* to affect bone mineral density.

Nishizawa et al.'s (2021) genome-wide association study (GWAS) aimed to identify the potential genetic variants that contribute to susceptibility to chronic pain and to the efficacy of administered drugs. Based on their GWAS, the 20 best candidate SNP sites were selected in patients with postherpetic neuralgia (PHN) for the rs9557470 SNP region in the *TMTC4* gene ($p = 0.00007228$) as patients with A/A = 23 (homozygote for the minor allele), A/B = 31 (heterozygote for the major allele), B/B = 35 (homozygote for the major allele) genotypes, where the controls had A/A = 24, A/B = 130, and B/B = 128.

Treutlein et al. (2009, as cited in Edenberg et al., 2010, p. 848) reported the results of a GWAS on German alcoholics. Hospitalized patients were male alcoholics treated or abstained from alcohol for a period of time. *TMTC4* gene rs753708 SNP results from Treutlein ($p = 0.000079$) were compared with the current GWAS regarding European Americans (EA; $p = .031$) and African Americans (AA; $p = .32$).

We explored the degree of linkage unevenness for these two SNP sites, which is usually expressed by the linkage disequilibrium (LD) coefficient D' and the correlation coefficient r^2. When compared with the literature data, the *TMTC4* gene's loci of $D' = 0.461501$, $r^2 = 0.106671$ (1000GENOMES, Phase_3, Kinh in Ho Chi Minh City, Vietnam [KHV]) and $D' = 0.507033$ and r^2=0.085205 (1000GENOMES, Phase_3, Sri Lankan Tamil in the UK [STU]) show rs9557470 and rs753708 to have full linkage equilibrium. The Ensembl linkage disequilibrium calculator determined rs763560019 and rs1484543954 as our SNP regions in the *TMTC4* gene to also lack linkage disequilibrium.

Li et al. (2018) found that disarming the widely expressed *TMTC4* gene in the mouse cochlea caused acquired hearing loss in mice. Their results showed this function by regulating *TMTC4* enriched in endoplasmic reticulum and its Ca^{2+} dynamics and unfolding protein response (UPR). Given the genetic link of UPR with hearing loss, this reveals a direct link between the more common NIHL and UPR. Our study showed T/T to be at 94% and T/A at 6% for the *TMTC4* gene SNP3- rs763560019 in the hearing loss group, while T/T was 99.4% and T/A 0.6% in the control group, with an $OR = 9.51$ (1.18-76.07). The tendency of T/A in *TMTC4* (T/A) to increase NIHL is statistically significant ($p = 0.01$).

Neurotrophins are endogenous molecules vital for nervous system development and neuronal morphogenesis. Brain-derived neurotrophic factor *(BDNF)* and neurotrophin 3 *(NTF3)* are highly expressed in internal hair cells and supporting cells to create precise synaptic connectivity in postpartum mice and to direct auditory nerve fibers to stabilize the connection in adult mice. Disabling *Ntf3* in supporting cells using the PLP1-CreER mouse line disrupts high-frequency hearing, while *BDNF* does not. On the contrary, excessive expression of *Ntf3* or *BDNF* in supporting cells and hair cells reduces both noise-related synaptic strip reduction and eventual hearing loss (Wan et al., 2014).

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BDNF and NTF3 have similar structures as members of the nerve growth factor (NGF) family of proteins. Chen et al. (2021) investigated the potential association of NIHL susceptibility with cochlear clock genes *(CRY1, CRY2, PER1, PER2), DNF* genes (brain-derived neurotrophic factor), and the *NTF3* gene among Chinese workers exposed to noise. The TaqMan-PCR technique was used to genotype *NTF3* rs1805149 (Hardy-Weinberg test $p = 0.592$). According to the results, the rs2585405*high temperature and rs6265*rs934945*rs1805149 models were associated with NIHL risk with *OR*s = 1.61 and 1.80, respectively ($p < 0.001$).

NTF3 may be a candidate gene for Alzheimer's disease (AD) due to the role it plays in neuroprotection and long-term memory formation. Liu et al. (2015) investigated the association of SNP regions in the *NTF3* gene (rs6489630 and rs6332) with AD in a Japanese population. They demonstrated the *NTF3* polymorphism rs6489630 to be a relevant risk factor for AD in this Chinese sample and a gene dose association to exist between the A allele of rs6332 and the onset of AD in ε4 non-carriers of apolipoprotein E4 *(ApoE4).*

Based on this, we evaluated the *NTF3* rs1805149 SNP region examined by Chen et al. (2021) and the *NTF3* rs6489630 and rs6332 SNP regions investigated by Liu et al. (2015) in terms of linkage disequilibrium (LD). According to the results, the difference (D') between the observed and the expected frequency of the given haplotype shows high linkage disequilibrium ($D' = 0.999959$). when the r2:0.070895 (1000GENOMES, Phase_3, Southern Han Chinese, China [CHS]) locus is in full linkage equilibrium with the *NTF3* gene's rs1805149 and rs6489630 SNP regions. On the other hand, when evaluating the association of the rs6332 A allele associated with the onset time of AD with NIHL using the Ensembl linkage disequilibrium calculator, we found rs1805149 and rs6332 the Gambian genome (as per the Gambian Genome Variation Project [GGVP], Gambian in Western Division [GWW]) to have moderate linkage disequilibrium $D' = 0.999996$, $r^2 = 0.546460$).

Park et al. (2014) revealed that the risk for emotional side effects regarding osmotic release oral system methylphenidate therapy in children with attention deficit/hyperactivity disorder may be associated with the *NTF3* rs6332 A allele. In line with this information, the *NTF3* rs6332 A allele can be said to have moderate linkage disequilibrium with NIHL regarding both Alzheimer's disease and attention deficit/hyperactivity disorder.

The genotype frequency of the *NTF3* gene at SNP6-rs1438314793 was found to have an *OR* = 1.40 (0.98-1.99) in the patient and control groups. Although SNP6 had a tendency to increase hearing loss, the result was not statistically significant ($p = 0.19$).

CNTN1 encodes the contactin 1 glycosylphosphatidylinositol-anchored neuronal membrane protein that acts as a cell adhesion molecule with vital roles in axonal development. Guerreiro et al. (2007) performed the first large-scale GWAS on dementia with Lewy bodies in a large cohort. Accordingly, *CNTN1*'s overlapping loci were also significant across the genome (rs79329964, *OR* = 1.5, $p = 0.000000435$). Rampersaud et al. (2014) aimed to identify type 2 diabetes susceptibility genes in the Amish with a GWAS and associated *CNTN1* rs2289522 with type 2 diabetes in the Amish ($p < 0.005$).

One recent study (Anderson et al., 2018) showed that variations in the *PLP1* gene, which encodes a myelin-related proteolipid, and in *CNTN1,* which encodes a protein related to axon guidance were associated with differences in interhemispheric integration. Anderson et al. performed *CNTN1* rs1056019 SNP genotyping using the neurite orientation distribution and density imaging (NODDI) technique to quantify axonal morphology in the subdivisions of the corpus callosum and

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linked them to genetic variations in *PLP1* and *CNTN1,* and these were found to be significantly correlated with the callosal microstructure.

*CNTN1'*s rs762307549 and rs745850349 do not have linkage disequilibrium in our study. Based on the data in the literature, these SNP regions have been determined not to have linkage disequilibrium with *CNTN1'*s rs762307549 and rs745850349 SNPs. In addition to the *NTF3* gene, the difference between the hearing loss and control groups was statistically significant ($p = 0.03$) for the genotype frequency regarding *CNTN1'*s SNP7-rs762307549 and SNP8-rs762307549.

This study determined the NIHL seen in the examined group to be related to a high noise environment and working time. This result was found to be consistent with the studies in the literature. The causal relationship between the effect of noise and hearing loss is clear, and the limiting the values determining the effects have been clearly determined. The organizational measures defined for protecting employees against noise consist of managerial and personal protectors that determine what work will be done according to noise level, including things such as source, engineering, environmental, and personal controls. As in many countries, however, while Turkey does have legal regulations, their implementation has shortcomings.

Various studies have shown the antioxidant system to become activated after exposure to noise, with noise exposure levels being important for activating this system. The current study has also shown people who work in high noise levels to be more likely to experience hearing loss. Therefore, the genetic polymorphisms specific to societies in this study differ from other studies where relationships have been found between genetic polymorphisms and NIHL. As a result, the antioxidant system is unlikely to behave the same at all times. However, when evaluating the sub-groups (i.e., noise exposure levels, noise exposure times) separately for oxidative stress levels and the exact relationship of proteins encoded by the relevant genes, a difference seems likely to occur (Hu & Deng, 2014).

As a result of this study, genetic variability in the *TAOK1, TMTC4* and *CNTN1* genes has been found to affect NIHL sensitivity in the Turkish population. In this regard, the study examined the rare SNP variants believed to be associated with hearing loss through the *TAOK1, TMTC4* and *CNTN1* genes that had not been previously included in the literature and found statistically significant relationships. This study found significant differences between the patient and control groups in terms of NIHL regarding the genotypes associated with five SNP regions of the *TAOK1, TMTC4,* and *CNTN1* genes, and the study results may suggest that these SNPs have a significant effect on noise sensitivity during noise exposure. The findings from this study also suggest that gene-environment interactions regarding exposure to environmental noise existed for all these SNP regions in the *TAOK1, TMTC4,* and *CNTN1* genes.

The sample size in our study was accepted based on a 0.80% allele frequency ($R_G = 2$), and we conducted the study over a total of 300 people, with 150 people in each group. According to the current findings, a power level of 0.33 remained with respect to the sample size, and this causes Type II errors to increase. As a result of the research, we determined allele frequencies at rates ranging between 96% and 99% and between 5% and 1%. According to these results, performing the post-hoc power analysis (probability of Type I errors = 0.05 for a power level = 0.80) and calculating the allele frequency as 96% ($R_G = 2$) reveals the fact that 525 individuals were included in each group and a total of 1,050 individuals were screened. While our study of SNP regions in previously unstudied genes does provide new contributions to the literature, it has the limitation of

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having a small sample size. However, the findings of this study provide new and potentially useful information for this NIHL population through the variants that are rare in the population. In addition, we were not able to check every possible lifestyle factor due to the observational nature of this design.

The size of the sample is heavily influenced by disease prevalence, allele frequency of the disease, linkage disequilibrium, inheritance patterns, and genetic variant effect sizes with regard to detecting associations between NIHL and SNP markers (Hong & Park, 2012).

The potential contribution of rare variants to disease risk in GWAS studies have been found to be of great interest. A genome-wide search for rare variations can be performed using sequencing technology over a large sample of patients and controls. A genome-wide analysis of the resulting data can be used to identify new risk genes for common disorders, as well as to assess the impact of rare genetic variations (Zöllner, 2012). Effective sample size refers to the minimum number of samples necessary in order to be able to achieve statistical power (Hong & Park, 2012).

Population-based studies with a large sample size have high statistical power and lead to smaller variance, but they accrue high costs and take a long time to reach a sufficient number of samples. Therefore, our study took these conditions into account and applied an analysis of rare variants with low minor allele counts. To overcome this limitation in our study, available genomic regions can be tested with rare variant burden methods by combining the default values of functional rare variants into aggregated statistics that are then compared between cases and controls.

A large sample size needs to be used in order to obtain adequate power when selecting case controls. The use of large sample sizes in variant studies is costly, so one alternative approach is to increase the frequency difference between cases and controls in order to increase the effect of the study. Using this method, individuals can be selected from the extremes of the phenotypic distribution, and tests can be applied based on their quantitative features. The effect as to the sizes of rare variants are expected to be higher than those of common variants in this context. In the case of rare variants, allelic heterogeneity modeling can be conducted as each locus will have multiple risk variants that may have different effects (Zöllner, 2012).

The preferred sample size varies for cases with regard to things such as the preferred modeling method, SNP type, and disease characteristics. The sample size per assumption is small in a dominant model, while a large number of samples are needed in a recessive model in order to achieve a sufficient level of statistical power. When testing for common SNPs with larger effect sizes, a smaller sample size is needed as the marker allele is more closely linked to the disease allele. Common diseases require a smaller sample size than rare diseases (Hong & Park, 2012).

The regression effect size of the variant and its prevalence in the population are inversely related in exome-wide and genome-wide sequencing studies. Rare SNP heritability is predicted to be impacted by missing heritability probabilities from genome-wide variants, which is consistent with assumptions about linkage disequilibrium, allelic frequency, and genotype precision (Bomba et al., 2017).

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As a result, some difficulties may arise in genetic association analysis studies of rare variants due to the presence of large amounts of candidate variants with low minor allele frequencies. Therefore, combining multiple variants will provide an opportunity for a gene-level association analysis and data can be obtained where information on disease heritability of rare variants is missing.

In this context, evaluating the combination of genetic variations with other risk factors is very important. NIHL is an occupational disease that has been proven to be affected by environmental factors. Numerous studies are found in terms of genetics that aim to focus on the increase or decrease of hearing loss. NIHL's environmental aspect appears more dominant in terms of occupational disease. Although several genetic predispositions for NIHL have been identified, oxidative stress genes are the candidate genes for NIHL. Various ethnic and racial groups display different distributions of genetic variations. Therefore, the results of genetic screening should be handled with special caution in order to avoid racial discrimination. Identifying at-risk employees may incorrectly strengthen the tendency to focus on this sub-population, while at the same time prevent many at-risk individuals from acknowledging that a combination of genetic, environmental, and behavioral risk factors are what lead to the development of NIHL.

Evaluating the characterization of occupational exposure together with screening tests will help to fully understand the role of genetic sensitivity in occupational diseases. Including this assessment routinely in screening programs in the workplace, however, will take a long time in terms of cost effectiveness and benefits (Öztan et al., 2020).

5. Conclusion

Genetic testing in the workplace carries many ethical and social risks that outweigh the perceived benefits. Difficulties in interpreting tests can lead to less effort placed into protection policies for reducing exposure in order to protect all employees from risks. Advances in laboratory and computer technologies have facilitated the study of the sensitivity of environmentally sensitive genes in relation to occupational diseases in recent years, with the focus being on the genetic polymorphism of candidate genes.

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6. References

Ahsan, M., & Waheed, F. (2020) Contribution of common single nucleotide polymorphisms in noise-induced hearing loss. *Pak-Euro Journal of Medical and Life Sciences*, *3*(3), 121–130.

Anderson, C., Gerding, W. M., Fraenz, C., Schlüter, C., Friedrich, P., Raane, M., Arning, L., Epplen, J. T., Güntürkün, O., Beste, C., Genç, E., & Ocklenburg, S. (2018). PLP1 and CNTN1 gene variation modulates the microstructure of human white matter in the corpus callosum. *Brain Structure and Function*, *223*(8), 3875–3887.

ACCEPTED UNEDITED MS

Bomba, L., Walter, K., & Soranz, N. (2017). The impact of rare and low-frequency genetic variants in common disease. *Genome Biology*, *18*(77), 1–17.

Carlsson, P. I., Laer, L. V. Borg, E., Bondeson, M. L., Thys, M., Fransen, E., & Camp, G. V. (2005). The influence of genetic variation in oxidative stress genes on human noise susceptibility. *Hearing Research*, *202*(1-2), 87–96.

Chang, N. C., Ho, C. K., Lin, H. Y., Yu, M. L., Chien, C. Y., & Ho, K. Y. (2011). Association of polymorphisms of heat shock protein 70 with susceptibility to noise-induced hearing loss in the Taiwanese population. *Audiology & Neurotology*, *16*(3), 168–174.

Chen, H., Ding, X., Ding, E., Chen, M., Wang, H., Yang, G., & Zhu, B (2021). A missense variant rs2585405 in clock gene PER1 is associated with the increased risk of noise-induced hearing loss in a Chinese occupational population. *BMC Med Genomics*, *14*(1), 221.

Chen, X. M., Xue, X. M., Yu, N., Guo, W. W., Yuan, S. L., Jiang, Q. Q., & Yang, S. M. (2022). The role of genetic variants in the susceptibility of noise-induced hearing loss. *Frontiers in Cellular Neuroscience*, *16*, 946206.

Davis, R. R., Newlander, J. K., Ling, X., Cortopassi, G. A., Krieg, E. F., & Erway, L. C. (2001). Genetic basis for susceptibility to noise-induced hearing loss in mice. *Hearing Research*, *155*(1-2), 82–90.

Edenberg, H. J., Koller, D. L., Xuei, X., Wetherill, L., McClintick, J. N., Almasy, L., Bierut, L. J., Bucholz, K. K., Goate, A., Aliev, F., Dick, D., Hesselbrock, V., Hinrichs, A., Kramer, J., Kuperman, S., Nurnberger, Jr., J. I., Rice, J. P., Schuckit, M. A., Taylor, R., Webb, B. T., Tischfield, J. A., Porjesz, B., & Foroud, T. (2010). Genome-wide association study of alcohol dependence implicates a region on chromosome 11. *Alcohol Clin Exp Res*. *34*(5), 840–852.

Eisenhaber, B., Sinha, S., Jadalanki, C. K., Shitov, V. A., Tan, Q. W., Sirota F. L., & Eisenhaber, F. (2021). Conserved sequence motifs in human TMTC1, TMTC2, TMTC3, and TMTC4, new O-mannosyltransferases from the GT-C/PMT clan, are rationalized as ligand binding sites. *Biology Direct*. *16*(4), 1–18.

Fang, C. Y., Lai, T. C., Hsiao, M., Chang, Y. C. (2020). The diverse roles of Tao kinases in health and diseases. *International Journal of Molecular Sciences*, *21*(20), 7463.

Gao, D., Wang, B., Sun, D., Wang, N., Guo, J., Zhang, S., & Zhu, B. (2020). Association of STAT3 gene polymorphism with noise-induced hearing loss (NIHL) in the Chinese population. *Research Square*. 1-24. https://doi:10.21203/rs.3.rs-41715/v1

Guerreiro, R., Ross, O. A., Kun-Rodrigues, C., Orme, T., Shpehard, C., Parkkinen, L., Darwent, L., Hernandez, D., Scholz, S., Ansorge, O., Clark, L., Honig, L. S., Marder, K., Clarimon, J., Lemstra, A., Scheltens, P., van der Flier, W., Louwersheimer, E., Holstege, H., …, Bras, J. (2007). Genome-wide association analysis of dementia with Lewy bodies reveals unique genetic architecture. *Nottingham Repository*. Retrieved from: https://nottingham-repository.worktribe.com/preview/908159/2017_DLB_GWAS_draft_for_circulation.pdf

Henderson, D., Bielefeld, E. C., Harris, K. C., & Hu, B. H. (2006). The role of oxidative stress in noise-induced hearing loss. *Ear and Hearing*, *27*(1), 1–19.

ACCEPTED UNEDITED MS

Henderson, D., Subramaniam, M., & Boettcher, F. A. (1993). Individual susceptibility to noise-induced hearing loss: an old topic revisited. *Ear and Hearing*, *14*(3), 152–168.

Hong, E. P & Park, J. W. (2012). Sample size and statistical power calculation in genetic association studies. *Genomics & Informatics*, *10*(2), 117–122.

Hu, L., & Deng, J. H. (2014). Progress in the association studies on susceptibility gene SNP for NIHL in human population. *Journal of Occupational Health*, *30*(18), 2664–2669.

Jiao, J., Yu, S., Gu, G., Chen, G., Zhang, H., & Zheng, Y. (2021). Relationship research between CDH23 gene and risk of noise-induced hearing loss. Research Square. 1-12. https://doi:10.3760/cma.j.issn.1001-9391.2020.02.002

Konings, A., Laer, L. V., & Camp, G. V. (2009). Genetic studies on noise-induced hearing loss: A review. *Ear and Hearing*, *30*(2), 151–159.

Kowalski, T. J., Pawelczyk, M., Rajkowska, E., Dudarewicz, A., & Sliwinska-Kowalska, M. (2014). Genetic variants of CDH23 associated with noise-induced hearing loss. *Otology & Neurotology*, *35*(2), 358–365.

Le Prell, C. G., Dolan, D. F., Schacht, J., Miller, J. M., Lomax, M. I., & Altschuler, R. A. (2003). Pathways for protection from noise induced hearing loss. *Noise Health*, *5*(20), 1–17.

Le Prell, C. G., Yamashita, D., Minami, S. B., Yamasoba, T., Miller, J. M. (2007). Mechanisms of noise-induced hearing loss indicate multiple methods of prevention. *Hearing Research*, *226*(1-2), 22–43.

Lei, S., Huang, L., Liu, Y., Xu, L., Wang, D., Yang, L. (2017). Association between polymorphisms of heat-shock protein 70 genes and noise-induced hearing loss: A meta-analysis. *PLoS One*, *12*(11), e0188539.

Li, J., Akil, O., Rouse, L. S., McLaughlin, W. C., Matthews, R. I., Lustig, R. L., Chan, K. D., & Sherr, H. E. (2018). Deletion of Tmtc4 activates the unfolded protein response and causes postnatal hearing loss. *Journal of Clinical Investigation*,*128*(11), 5150–5162.

Li, X., Zhu, Z., Li, W., Wei, L., Zhao, B., Hao, Z. (2020). Polymorphism in GRHL2 gene may contribute to noise-induced hearing loss susceptibility: A meta-analysis. *Brazilian Journal of Otorhinolaryngology*, *86*(3), 370–375.

Liu, M., Huo, Y. R., Wang, J., Liu, S., Liu, S., Wang, C., Wang, J., & Ji, Y. (2015). Polymorphisms of the neurotrophic factor-3 (NTF-3) in Alzheimer's disease: Rs6332 associated with onset time and rs6489630 T allele exhibited a protective role. *Journal of Neurogenetics*, *29*(4), 183–187.

Liu, Y. M., Li, X. D., Guo, X., Liu, B., Lin, A. H., & Rao, S. Q. (2010). Association between polymorphisms in SOD1 and noise-induced hearing loss in Chinese workers. *Acta Otolaryngologica*, *130*(4), 477–486.

Meisinger, C., Prokisch, H., Gieger, C., Soranzo, N., Mehta, D., Rosskopf, D., Lichtner, P., Klopp, N., Stephens, J., Watkins, N. A., Deloukas, P., Greinacher, A., Koenig, W., Nauck, M., Rimmbach, C., Völzke, H., Peters, A., Illig, T., Ouwehand, W. H., Meitinger, T., Wichmann, H.-Erich, & Döring, A. (2009). A genome-wide association study identifies three loci associated with mean platelet volume. *American Journal of Human Genetics*, *84*(1), 66–71.

ACCEPTED UNEDITED MS

Miao, L., Ji, J., Wan, L., Zhang, J., Yin, L., & Pu, Y. (2019). An overview of research trends and genetic polymorphisms for noise-induced hearing loss from 2009 to 2018. *Environmental Science and Pollution Research*, *26*(34), 34754–34774.

Münzel, T., Steven, S., Frenis, K., Lelieveld, J., Hahad, O., & Daiber, A. (2020). Environmental factors such as noise and air pollution and vascular disease. *Antioxidants & Redox Signaling*, *33*(9), 581–601.

Nelson, D. I., Nelson, R. Y., Concha-Barrientos, M., & Fingerhut, M. (2005). The global burden of occupational noise-induced hearing loss. *American Journal of Industrial Medicine*, *48*(6), 446–458.

Nishizawa, D., Iseki, M., Arita, H., Hanaoka, K., Yajima, C., Kato, J., Ogawa, S., Hiranuma, A., Kasai, S., Hasegawa, J., Hayashida, M., & Ikeda, K. (2021). Genome-wide association study identifies candidate loci associated with chronic pain and postherpetic neuralgia. *Molecular Pain*, *17*, 1–21.

Ohlemiller, K. K., Wright, J. S., & Dugan, L. L. (1999). Early elevation of cochlear reactive oxygen species following noise exposure. *Audiology and Neurotology*, *4*(5), 229–236.

Öztan, G., & İşsever, H. (2020). Molecular mechanisms of genetic polymorphisms associated with occupational diseases. *Journal of Istanbul Faculty of Medicine*, *83*(2), 152–161.

Park, S., Kim, B. N., Kim, J. W., Shin, M. S., Cho, S. C., Kim, J. H., Son, J. W., Shin, Y. M., Chung, U. S., & Han, D. H. (2014). Neurotrophin 3 genotype and emotional adverse effects of osmotic-release oral system methylphenidate (OROS-MPH) in children with attention-deficit/hyperactivity disorder. *Journal of Psychopharmacology*, *28*(3), 220–226.

Rabinowitz, P. M., Wise, Sr., J. P., Mobo, B. H., Antonucci, P. G., Powell, C., & Slade, M. (2002). Antioxidant status and hearing function in noise-exposed workers. *Hearing Research*, *173*(1-2), 164–171.

Rampersaud, E., Damcott, C. M., Fu, M., Shen, H., McArdle, P., Shi, X., Shelton, J., Yin, J., Chang, Y. P. C., Ott, S. H., Zhang, L., Zhao, Y., Mitchell, B. D., O'Connell, J., & Shuldine, A. R (2007). Identification of novel candidate genes for type 2 diabetes from a genome-wide association scan in the Old Order Amish: evidence for replication from diabetes-related quantitative traits and from independent populations. *Diabetes*, *56*(12), 3053–3062.

Reddy, S. T., Wadleigh, D. J., Grijalva, V., Ng, C., Hama, S., Gangopadhyay, A., Shih, S. M., Lusis, A. J., Navab, M., & Fogelman, A. M. (2001). Human paraoxonase-3 is an HDL-associated enzyme with biological activity similar to paraoxonase-1 protein but is not regulated by oxidized lipids. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *21*(4), 542–547.

Republic of Turkey Ministry of Labor and Social Security General Directorate of Occupational Health and Safety. (2012). *Health surveillance guide at work* (p. 52). Author.

Shen, H., Huo, X., Liu, K., Li, X., Gong, W., Zhang, H., Xu, Y., Wang, M., Li, X., Zhang, J., Zhang, Z., & Zhu, B. (2012). Genetic variation in GSTM1 is associated with susceptibility to noise-induced hearing loss in a Chinese population. *Journal of Occupational and Environmental Medicine*, *54*(9), 1157–1162.

ACCEPTED UNEDITED MS

Tessier-Sherman, B., Galusha, D., Cantley, L. F., Cullen, M. R., Rabinowitz, P. M., & Neitzel, R. L. (2017). Occupational noise exposure and risk of hypertension in an industrial workforce. *American Journal of Industrial Medicine*, *60*(12), 1031–1038.

Themann, L.C., & Masterson, A. E. (2019). Occupational noise exposure: A review of its effects, epidemiology, and impact with recommendations for reducing its burden. *Journal of the Acoustical Society of America*, *146*(5), 3879–3905.

Wan, G., Gómez-Casati, M. E., Gigliello, A. R., Liberman, M. C., & Corfas, G. (2014). Neurotrophin-3 regulates ribbon synapse density in the cochlea and induces synapse regeneration after acoustic trauma. *Elife*, *3*, e03564.

Wang, B., Han, L., Dai, S., Li, X., Cai, W., Yang, D., Chen, L., Wang, N., Zhu, B., & Zhang, J. (2018). Hearing loss characteristics of workers with hypertension exposed to occupational noise: A cross-sectional study of 270,033 participants. *BioMed Research International*, *2018*, 8541638.

White, C. H., Ohmen, J. D., Sheth, S., Zebboudj, A. F., McHugh, R. K., Hoffman, L. F., Lusis, A. J., Davis, R. C., & Friedman, R. A. (2009). Genome-wide screening for genetic loci associated with noise-induced hearing loss. *Mammalian Genome*, *20*(4), 207–213.

Zhang, S., Ding, E., Yin, H., Zhang, H, & Zhu, B. (2019). Research and Discussion on the relationships between noise-induced hearing loss and ATP2B2 gene polymorphism. *International Journal of Genomics*, *2019*(5048943), 1–8.

Zhang, X., Liu, Y., Zhang, L., Yang, Z., Yang, L., Wang, X., Jiang, C., Wang, Q., Xia, Y., Chen, Y., Wu, O., & Zhu, Y. (2015). Associations of genetic variations in EYA4, GRHL2 and DFNA5 with noise-induced hearing loss in Chinese population: a case-control study. *Environmental Health*, *14*, 77.

Zheng, H. Cui., W., & Yan, Z. (2021). Ion channel-related hereditary hearing loss: A narrative review. *Journal of Bio-X Research*, *4*(4), 145–150.

Zöllner, S. (2012). Sampling strategies for rare variant tests in case-control studies. *European Journal of Human Genetics*, *20*(10), 1085–1091.

ACCEPTED UNEDITED MS